

Lignicolous freshwater ascomycetes from Thailand: Introducing *Dematiopyriforma muriformis* sp. nov., one new combination and two new records in Pleurotheciaceae

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Abstract

During the study of lignicolous freshwater fungi from Thailand, three pleurotheciaceous species were collected from freshwater habitats in Thailand. Two were identified as *Pleurothecium aquaticum* and *Rhexoacrodictys fimicola*, and the third is a new species *Dematiopyriforma muriformis* sp. nov.. *Rhexoacrodictys* is accepted in Pleurotheciaceae based on phylogenetic analysis. *Rhexoacrodictys nigrospora* is transferred to *Dematiopyriforma* based on phylogenetic analysis and morphological characters. *Pleurothecium aquaticum* and *Rhexoacrodictys fimicola* are reported from Thailand for the first time.

Keywords

1 new combination, 1 new taxon, freshwater fungi, phylogeny, Pleurotheciales, taxonomy

Introduction

Pleurotheciales was introduced by Réblová et al. (2016) to accommodate a single family Pleurotheciaceae. The order was originally placed in Hypocreomycetidae by Réblová et al. (2016). Hongsanan et al. (2017) showed that Pleurotheciales clustered with Conioscyphales, Fuscosporellales and Savoryellales in a monophyletic clade within Sordariomycetes. Hence, they transferred Pleurotheciales to a newly introduced subclass Savoryellomycetidae based on phylogenetic analysis and the placement has been confirmed and accepted by Dayarathne et al. (2019) and Hyde et al. (2020a).

Pleurotheciaceae was introduced by Réblová et al. (2016) with *Pleurothecium* Höhn. as the type genus. Currently, *Adelosphaeria*, *Anapleurothecium*, *Coleodictyospora*, *Dematiapyriforma*, *Helicoascotaiwania*, *Melanotrigonum*, *Neomonodictys*, *Phaeoisaria*, *Phragmocephala*, *Pleurotheciella*, *Pleurothecium*, *Saprodesmium*, and *Sterigmatobotrys* are accepted in this family (Hyde et al. 2020a; Wijayawardene et al. 2020; Dong et al. 2021). The sexual morphs of Pleurotheciaceae share dark, papillate, perithecial, astromatic, immersed to superficial ascomata, unitunicate asci with a distinct non-amyloid apical annulus, and fusiform to ellipsoidal, septate, hyaline ascospores (Réblová et al. 2016; Luo et al. 2018a; Hyde et al. 2020a). The asexual morphs of Pleurotheciaceae are diverse in morphology, comprising *acrodictys*-like (*Monotosporella*), (Hyde and Yanna 2002; Sadowski et al. 2012), *helicoön*-like (*Helicoascotaiwania*, Dayarathne et al. 2019; Réblová et al. 2020), *monodictys*-like (*Neomonodictys*, Hyde et al. 2020b) and *dactylaria*-like taxa (*Pleurotheciella*, *Phaeoisaria* and *Pleurothecium*, Réblová et al. 2016; Luo et al. 2018a). Species in Pleurotheciaceae are cosmopolitan with a worldwide distribution and have been reported from both aquatic and terrestrial habitats (Réblová et al. 2016, 2020; Hernandez-Restrepo et al. 2017; Luo et al. 2018a, 2019; Hyde et al. 2020a, b).

In this study, three new collections are placed in *Dematiapyriforma*, *Rhexoacrodictys* and *Pleurothecium* respectively. The monotypic genus *Dematiapyriforma* was introduced to accommodate an endophytic species, *D. aquilaria* which was collected from wood of *Aquilaria crassna* (Sun et al. 2017). *Dematiapyriforma* was originally placed in Savoryellales (Sun et al. 2017). However, Dong et al. (2021) showed that *Dematiapyriforma* clustered within Pleurotheciales and sister to *Rhexoacrodictys* and *Saprodesmium*. In addition, the morphology of *Dematiapyriforma* is similar to *Neomonodictys* in Pleurotheciales. Therefore, they transferred *Dematiapyriforma* to Pleurotheciales based on phylogenetic analysis and morphological characteristics. *Rhexoacrodictys* was introduced by Baker et al. (2002) to accommodate species previously identified as *Acorcdictys* (i.e., *A. erecta*, *A. fimicola*, *A. fuliginosa* and *A. queenslandica*) and wherein *Rhexoacrodictys erecta* was designated as the type. Two additional species *R. martini* and *R. broussonetiae* were subsequently added to the genus based on morphological characteristics (Delgado 2009; Xiao et al. 2018). While *R. martini* and *R. queenslandica* were transferred to *Distoseptispora* and *Junewangia* based on phylogenetic analysis (Xia et al. 2017). Currently, four species are accepted in *Rhexoacrodictys*. *Pleurothecium* was established by Höhnelt (1919) with

P. recurvatum (Morgan) Höhn as type species. *Pleurothecium* species are characterized by macronematous, mononematous, septate, brown conidiophores, polyblastic, sympodially extended, denticulate conidiogenous cells and solitary, septate, hyaline or pigmented or bicolored conidia (Goos 1969; Matsushima 1975, 1980; Subramanian and Bhat 1989; Matsushima and Matsushima 1996; Cooper 2005; Arzanlou et al. 2007; Wu and Zhang 2009; Réblová et al. 2012; Monteiro et al. 2016; Luo et al. 2018a). Presently, 11 species are accepted in the genus. Most *Pleurothecium* species are reported as saprobes from freshwater or terrestrial habitats (Wu and Zhang 2009; Réblová et al. 2012; Monteiro et al. 2016; Luo et al. 2018a).

We are currently investigating the diversity of lignicolous freshwater fungi from the Greater Mekong Subregion (Hyde et al. 2016). Thailand is an area of the Greater Mekong Subregion with rich fungal biodiversity. Freshwater fungi have been studied in Thailand over several decades initiated by Tubaki et al. (1983) who found 40 Ingoldian fungi in the stream foams. Many new freshwater taxa have since been reported in Thailand, especially a large number of lignicolous freshwater ascomycetes (Sivichai et al. 1998, 2000, 2002; Jones et al. 1999; Sivichai and Boonyene 2004; Zhang et al. 2011; Luo et al. 2019; Dong et al. 2020; Calabon et al. 2021, 2022). Until 2020, more than 302 freshwater taxa had been reported from Thailand (Zhang et al. 2011; Calabon et al. 2021). In this study, we introduce three taxa of Pleurotheciaceae, collected from freshwater habitats in Thailand. With phylogenetic analysis of ITS, LSU, SSU, RPB2 and TEF1- α sequence data, they are placed in *Dematiopyriforma*, *Pleurothecium* and *Rhexoacrodictys* within Pleurotheciaceae. Of these three species, one is identified as *Pleurothecium aquaticum*, one as *Rhexoacrodictys fimicola*, and the third as a new species in *Dematiopyriforma*. In addition, *Rhexoacrodictys nigrospora* is transferred to *Dematiopyriforma* based on morphological and phylogenetic evidence.

Materials and methods

Collection, isolation and morphology

Submerged decaying woods were collected from the streams in Thailand. The sample incubation, examination and morphological studies were referred to the methods described by Luo et al. (2018b). Single spore isolations were followed the methods outlined by Senanayake et al. (2020). Specimens (dry wood with fungal material) were deposited in the herbarium of Mae Fah Luang University (**MFLU**), Chiang Rai, Thailand and Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (**KUN-HKAS**). Pure cultures were deposited in Mae Fah Luang University Culture Collection (**MFLUCC**) and Kunming Institute of Botany culture collection (**KUNCC**). Faces of Fungi and Index Fungorum numbers were registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2022). The descriptions are added to it GMS database (Chaiwan et al. 2021).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal mycelium (*Rhexoacrodictys erecta* and *Pleurothecium aquaticum*) or directly from the conidiamatal tissue thalli of fungi (*Dematiopyriforma muriformis*) as outlined by Wanasinghe et al. (2018). The Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, China) was used to extract DNA following the manufacturer's instructions. ITS, LSU, SSU, RPB2 and TEF1- α gene regions were amplified using the primer pairs ITS5/ITS4, LR0R/LR7, NS1/NS4, fRPB2-5F/fRPB2-7cR and 983F/2218R, respectively (Vilgalys and Hester 1990; White et al. 1990; Liu et al. 1999). The amplification was performed in a 25 μ l reaction volume containing 9.5 μ l ddH₂O, 12.5 μ l 2 $\times$ Taq PCR Master Mix with blue dye (Sangon Biotech, China), 1 μ l of DNA template and 1 μ l of each primer (10 μ M). The amplification condition for ITS, LSU, SSU, RPB2 and TEF1- α were followed Luo et al. (2018b). DNA sequencing of PCR products were carried out using the above-mentioned PCR primers at Tsingke Biological Engineering Technology and Services Co. (Yunnan, P.R. China).

Phylogenetic analyses

The taxa used in the phylogenetic analysis were obtained from previous studies (Table 1) (Hernandez-Restrepo et al. 2017; Luo et al. 2018a, 2019; Dayarathne et al. 2019; Hyde et al. 2020b; Réblová et al. 2020; Boonmee et al. 2021; Dong et al. 2021) and downloaded from GenBank. SEQMAN v. 7.0.0 (DNASTAR, Madison, WI) was used to assemble the consensus sequences and MAFFT v.7 online program (<http://mafft.cbrc.jp/alignment/server/>) was used to align the sequences (Kato et al. 2019). BioEdit was used to manually adjust the alignments and the alignment fasta file was converted to Phylip format by Aliview (Hall 2021; Larsson 2014).

Maximum likelihood (ML) analysis generated using the RAxML-HPC2 on XSEDE (v.8.2.8) in the CIPRES Science Gateway (<https://www.phylo.org>, Stamatakis 2006; Stamatakis et al. 2008; Miller et al. 2010) with rapid bootstrap analysis, followed by 1000 bootstrap replicates, using the GTR+I+G model of evolution.

Bayesian analysis was performed by MrBayes v. 3.2 (Ronquist et al. 2012), best-fit model of DNA evolution for the Bayesian inference analysis was estimated by MrModeltest v. 2.2 (Nylander 2004) and the GTR+I+G model was selected for LSU, ITS, RPB2 and TEF1- α , GTR+G model was selected for SSU. Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) was defined by Bayesian Markov Chain Monte Carlo (BMCMC) sampling method in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov Chains were run for 50,000,000 generations and trees were sampled every 500th generation (resulting in 100,000 trees). The first 20% trees that represented the burn-in phase were discarded and the remaining 80% (post burn-in) trees used for calculating posterior probabilities (PP) for the majority rule consensus tree.

Phylogenetic trees were visualized with FigTree v. 1.4.2 (Rambaut 2014) and edited in Microsoft Office PowerPoint 2019 (Microsoft Inc., United States). Newly generated sequences in this study were deposited in GenBank.

Table 1. Taxa used in this study; the ex-type strains were indicated in bold, newly generated sequences are indicated by * after the species name.

Species	Strain number	GenBank accession number				
		ITS	LSU	SSU	RPB2	TEF1- α
<i>Adelosphaeria catenata</i>	CBS 138679	KT278721	KT278707	KT278692	KT278743	–
<i>Anapleurothecium botulisporum</i>	CBS 132713	KY853423	KY853483	–	–	–
<i>Ascotaiwania lignicola</i>	NIL00005	HQ446341	HQ446364	HQ446284	HQ446419	HQ446307
<i>Ascotaiwania sawadae</i>	SS00051	HQ446340	HQ446363	HQ446283	HQ446418	HQ446306
<i>Bactrodesmiastrum obovatum</i>	FMR 6482	FR870264	FR870266	–	–	–
<i>Bactrodesmiastrum pyriforme</i>	FMR 10747	FR870263	FR870265	–	–	–
<i>Bactrodesmium abruptum</i>	CBS 144404	MN699391	MN699408	MN699365	MN704288	MN704313
<i>Bactrodesmium leptopus</i>	CBS 144542	MN699388	MN699423	MN699374	MN704297	MN704321
<i>Bactrodesmium obovatum</i>	CBS 144077	MN699395	MN699424	MN699375	MN704298	MN704322
<i>Canalisporium exiguum</i>	SS00809	GQ390296	GQ390281	GQ390266	HQ446436	–
<i>Canalisporium grenadoideum</i>	SS03615	–	GQ390267	GQ390252	HQ446420	HQ446309
<i>Coleodictyospora muriformis</i>	MFLUCC 18–1243	MW981642	MW981648	MW981704	–	–
<i>Coleodictyospora muriformis</i>	MFLUCC 18–1279	MW981643	MW981649	MW981705	–	–
<i>Conioscypha hoehnelii</i>	FMR 11592	KY853437	KY853497	HF937348	–	–
<i>Conioscypha lignicola</i>	CBS 335.93	–	AY484513	JQ437439	JQ429260	–
<i>Conioscypha peruviana</i>	ILL41202	–	KF781539	–	–	–
<i>Conioscypha pleiomorpha</i>	FMR 13134	KY853438	KY853498	–	–	–
<i>Dematiapyriforma aquilaria</i>	CGMCC 3.17268	KJ138621	KJ138623	KJ138622	–	–
<i>Dematiapyriforma muriformis</i> *	MFLU 21–0146	OM654773	OM654770	–	–	OM672032
<i>Dematiapyriforma nigrospora</i>	MFLUCC 21–0096	MZ538524	MZ538558	–	–	MZ567100
<i>Dematiapyriforma nigrospora</i>	MFLUCC 21–0097	MZ538525	MZ538559	MZ538574	MZ567113	MZ567101
<i>Fuscosporella pyriformis</i>	MFLUCC 16–0570	–	KX550896	KX550900	KX576872	–
<i>Helicoascotaiwania farinosa</i>	ILLS 53605	–	AY094189	–	–	–
<i>Helicoascotaiwania farinosa</i>	DAOMC 241947	JQ429145	JQ429230	–	–	–
<i>Helicoascotaiwania lacustris</i>	CBS 145963	–	MN699430	MN699382	MN704304	MN704329
<i>Helicoascotaiwania lacustris</i>	CBS 145964	MN699400	MN699431	MN699383	MN704305	–
<i>Helicoascotaiwania lacustris</i>	CBS 146144	MN699401	MN699432	MN699384	MN704306	–
<i>Leotia lubrica</i>	AFTOL-ID1	DQ491484	AY544644	AY544746	DQ470876	DQ028596
<i>Melanotrigonum ovale</i>	CBS 138815	KT278722	KT278711	KT278698	KT278747	–
<i>Microglossum rufum</i>	AFTOL-ID 1292	–	DQ470981	DQ471033	DQ470933	DQ471104
<i>Monotosporella setosa</i>	HKUCC3713	–	AF132334	–	–	–
<i>Mucispora obscuriseptata</i>	MFLUCC 15–0618	–	KX550892	KX550897	–	–
<i>Mucispora phangngaensis</i>	MFLUCC 16–0865	–	MG388210	MG388207	–	–
<i>Neomonodictys muriformis</i>	MFLUCC 16–1136	MN644509	MN644485	–	–	MN646856
<i>Obliquifusoidium guttulatum</i>	MFLUCC 18–1233	MW981645	MW981650	MW981706	–	–
<i>Parafuscosporella garethii</i>	FF00725.01	–	KX958430	KX958428	KX958432	–
<i>Parafuscosporella moniliformis</i>	MFLUCC 15–0626	–	KX550895	KX550899	–	–
<i>Parafuscosporella mucosa</i>	MFLUCC 16–0571	–	MG388211	MG388208	–	–
<i>Phaeoisaria aquatica</i>	MFLUCC 16–1298	MF399237	MF399254	–	MF401406	–
<i>Phaeoisaria clematidis</i>	MFLUCC 17–1968	MG837022	MG837017	MG837027	–	–
<i>Phaeoisaria fasciculata</i>	CBS 127885	–	KT278705	KT278693	KT278741	–
<i>Phaeoisaria filiformis</i>	MFLUCC 18–0214	MK878381	MK835852	MK834785	–	MN200285
<i>Phaeoisaria guttulata</i>	MFLUCC 17–1965	MG837021	MG837016	MG837026	–	–
<i>Phaeoisaria pseudoclematidis</i>	MFLUCC 11–0393	–	KP744501	KP753962	–	–
<i>Phaeoisaria sedimenticola</i>	CGMCC 3.14949	–	JQ031561	–	–	–
<i>Phaeoisaria sedimenticola</i>	S-908	MK878380	MK835851	–	–	MN200284
<i>Phaeoisaria sparsa</i>	FMR11939	–	HF677185	–	–	–
<i>Phragmocephala stemphylioides</i>	DAOM 673211	KT278730	KT278717	–	–	–
<i>Pleurotheciella aquatica</i>	MFLUCC 17–0464	MF399236	MF399253	MF399220	MF401405	–
<i>Pleurotheciella centenaria</i>	DAOM 229631	–	JQ429234	JQ429246	JQ429265	–
<i>Pleurotheciella fusiformis</i>	MFLUCC 17–0115	MF399232	MF399249	MF399217	MF401402	–
<i>Pleurotheciella guttulata</i>	KUMCC 15–0296	MF399240	MF399257	MF399223	MF401409	–
<i>Pleurotheciella krabiensis</i>	MFLUCC 18–0852	MG837018	MG837013	MG837023	–	–

Species	Strain number	GenBank accession number				
		ITS	LSU	SSU	RPB2	TEF1- α
<i>Pleurotheciella lunata</i>	MFLUCC 17–0111	MF399238	MF399255	MF399221	MF401407	–
<i>Pleurotheciella rivularia</i>	CBS 125238	–	JQ429232	JQ429244	JQ429263	–
<i>Pleurotheciella rivularia</i>	CBS 125237	–	JQ429233	JQ429245	JQ429264	–
<i>Pleurotheciella saprophytica</i>	MFLUCC 16–1251	MF399241	MF399258	MF399224	MF401410	–
<i>Pleurotheciella submersa</i>	MFLUCC 17–1709	MF399243	MF399260	MF399226	MF401412	–
<i>Pleurotheciella submersa</i>	MFLUCC 17–0456	MF399244	MF399261	MF399227	MF401413	–
<i>Pleurotheciella tropica</i>	MFLUCC 16–0867	MG837020	MG837015	MG837025	–	–
<i>Pleurotheciella uniseptata</i>	DAOM 673210	KT278729	KT278716	–	–	–
<i>Pleurothecium aquaticum</i>	MFLUCC 17–1331	MF399245	MF399263	–	–	–
<i>Pleurothecium aquaticum</i> *	KUMCC 21-0477	OM654775	OM654772	OM654807	OM672034	OM672033
<i>Pleurothecium floriforme</i>	MFLUCC 15–0628	NR_156614	NG_059791	–	–	–
<i>Pleurothecium obovoideum</i>	CBS 209.95	EU041784	EU041841	–	–	–
<i>Pleurothecium pulneyense</i>	MFLUCC 16–1293	–	MF399262	MF399228	MF401414	–
<i>Pleurothecium recurvatum</i>	CBS 138686	–	KT278715	KT278702	–	–
<i>Pleurothecium semifecundum</i>	CBS 131271	–	JQ429240	JQ429254	JQ429270	–
<i>Rhexoacrodictys erecta</i>	HSAUPmyr4622	KU999964	KX033556	KX033526	–	–
<i>Rhexoacrodictys erecta</i>	IFRD500–016	MT555421	MT5559123	MT555735	–	–
<i>Rhexoacrodictys erecta</i>	HSAUP myr6489	KU999963	KX033555	KX033525	–	–
<i>Rhexoacrodictys fimicola</i>	HMAS 47737	KU999960	KX033553	KX033522	–	–
<i>Rhexoacrodictys fimicola</i>	HMAS 42882	KU999962	KX033554	KX033524	–	–
<i>Rhexoacrodictys fimicola</i>	HMAS 43690	KU999957	KX033550	KX033519	–	–
<i>Rhexoacrodictys fimicola</i> *	MFLUCC 18–0340	OM654774	OM654771	OM654806	–	–
<i>Saprodesmium dematiosporium</i>	KUMCC 18–0059	MW981646	MW981647	MW981707	–	–
<i>Savoryella aquatica</i>	SS03801	–	HQ446372	HQ446292	HQ446405	HQ446326
<i>Savoryella lignicola</i>	NF00204	–	HQ446378	HQ446300	HQ446413	HQ446334
<i>Sterigmatobotrys macrocarpa</i>	MR2973	–	GU017317	–	–	–
<i>Sterigmatobotrys rudis</i>	DAOM 229838	JQ429152	JQ429241	JQ429256	JQ429272	–
<i>Sterigmatobotrys uniseptata</i>	MFLUCC 15–0358	MK878379	MK835850	MK834784	–	–

Results

Phylogenetic analyses

The dataset of combined ITS, LSU, SSU, RPB2 and TEF1- α sequence data comprises 81 strains with 4257 characters including gaps (ITS: 509 bp, LSU: 1006 bp, SSU: 862 bp, RPB2: 1032 bp, TEF1- α : 848 bp). *Leotia lubrica* (AFTOL-ID1) and *Microglossum rufum* (AFTOL-ID 1292) were used as outgroup taxa. RAxML and Bayesian analyses were conducted and resulted in generally congruent topologies. The best RAxML tree with a final likelihood value of –45872.924927 is presented. The matrix had 2433 distinct alignment patterns, with 44.65% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.234712, C = 0.261626, G = 0.290634, T = 0.213028; substitution rates AC = 1.347806, AG = 2.754719, AT = 1.490447, CG = 1.095887, CT = 6.696475, GT = 1.000000; gamma distribution shape parameter α = 0.316898.

In the phylogenetic analysis, *Dematiapyriforma muriformis* (MFLU 21–0146) clustered with the ex-type strain of *D. aquilaria* (CGMCC 3.17268) with low support (Fig. 1). The new isolate of *Rhexoacrodictys fimicola* (MFLUCC 18–0340) clustered

with three strains of *R. fimicola* (HMAS 42882, HMAS 43690 and HMAS 47737) with 100% ML/1.00 PP support (Fig. 1). *Pleurothecium aquaticum* (KUNCC 21–0477) clustered with the ex-type strain of *P. aquaticum* (MFLUCC 17–1331) with 100% ML/1.00 PP support (Fig. 1).

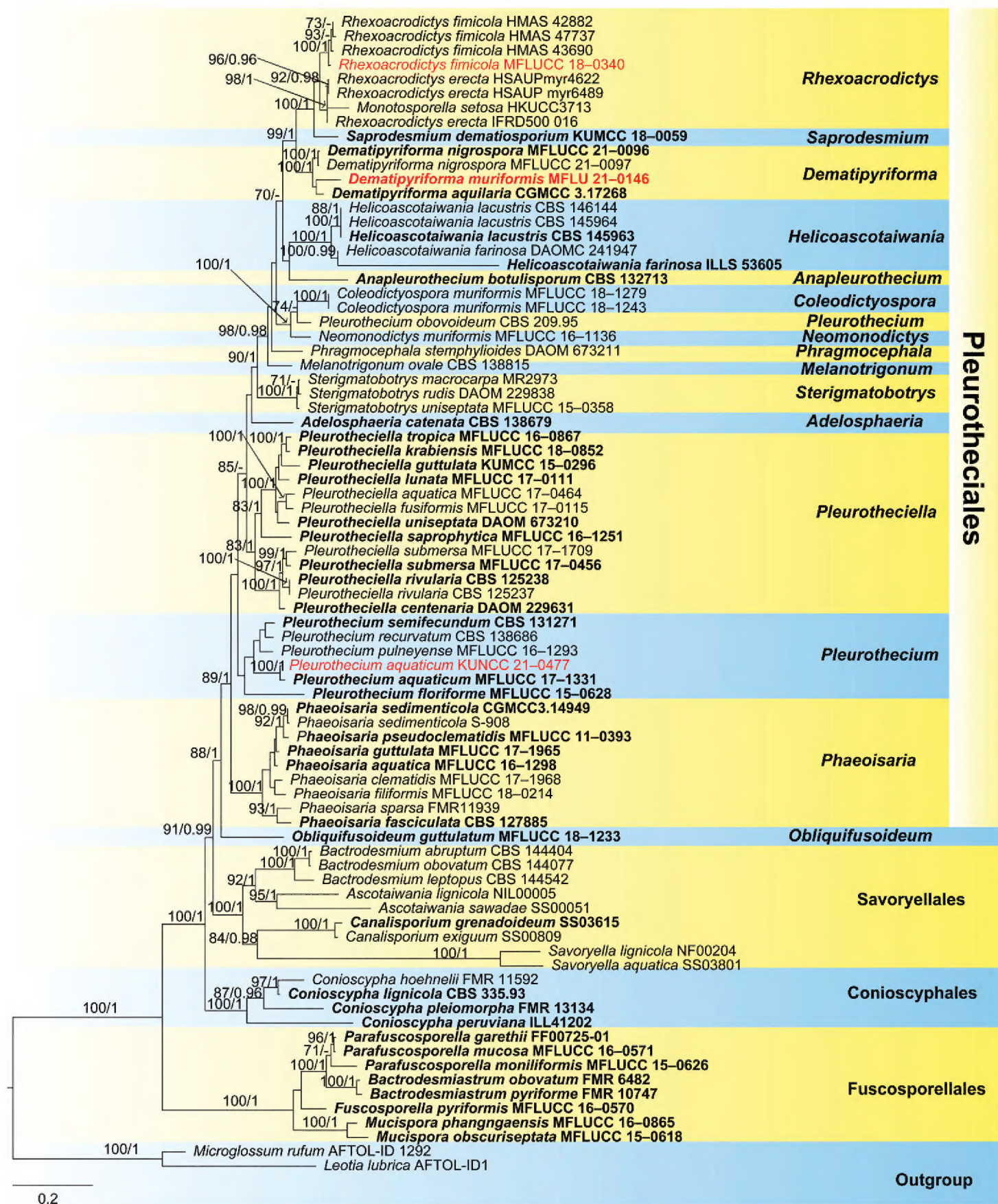


Figure 1. Phylogram based on a combined ITS, LSU SSU, RPB2 and TEF1- α sequence data of selected members of four orders of the Savioryellomycetidae. Bootstrap support values for maximum likelihood (ML) greater than 70% and Bayesian posterior probabilities (PP) greater than 0.95 are given as ML/PP above the nodes. Newly obtained sequences are indicated in red and ex-type strains are in bold.

Taxonomy

***Dematipyriforma muriformis* D.F. Bao, K.D. Hyde & Z.L. Luo, sp. nov.**

Index Fungorum Number: IF553383

Facesoffungi Number: FoF10414

Fig. 2

Etymology. Referring to the muriform conidia of this species.

Holotype. MFLU 21–0146.

Description. *Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on substratum superficial, scattered, black, shining, granulate. *Mycelium* immersed, composed of hyaline, branched, septate, smooth, hyphae. *Conidiomata* sporodochial, subhyaline. *Conidiophores* $10\text{--}26.5 \times 2\text{--}3 \mu\text{m}$ ($\bar{x} = 18.2 \times 2.3 \mu\text{m}$, $n = 20$), micronematous to semi-macronematous, mononematous, fasciculate, simple or branched, hyaline, cylindrical, smooth. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, hyaline, smooth. *Conidia* $23\text{--}26 \times 15.5\text{--}18 \mu\text{m}$ ($\bar{x} = 24.6 \times 16.7 \mu\text{m}$, $n = 30$), acrogenous, solitary, smooth, thick-walled, ellipsoidal to obovoid, muriform, rounded at apex, pointed at base, with 3–5 transverse septa, 1-longitudinal septum in all cells and rarely in end cells, slightly constricted at septa, subhyaline to pale olivaceous when young, olive to dark brown at maturity.

Material examined. THAILAND, Bangkok Province, Bang Kapi District, on decaying wood submerged in a freshwater stream, 3 October 2017, Z.L. Luo, Bsite 4–3–1 (MFLU 21–0146, holotype; KUN-HKAS 122858, isotype).

Notes. In the phylogenetic analysis, *Dematipyriforma muriformis* clustered with the ex-type strain of *D. aquilaria* (CGMCC 3.17268) within Pleurotheciaceae with low support (Fig. 1). The ITS blast result in NCBI GenBank showed that *D. muriformis* (MFLU 21–0146) is 92.36% and 91.92% similar to *D. nigrospora* (MFLUCC 21-0097) and *D. aquilaria* (CGMCC 3.17268) respectively.

Dematipyriforma muriformis resembles *D. aquilaria* in having micronematous, mononematous, smooth septate conidiophores, monoblastic, integrated, terminal, determinate conidiogenous cells and solitary, muriform conidia. However, *D. muriformis* differs from *D. aquilaria* in having hyaline conidiophores and slightly smaller conidia ($23\text{--}26 \times 15.5\text{--}18$ vs. $25\text{--}37.5 \times 15\text{--}22.5 \mu\text{m}$). In addition, conidia of *D. muriformis* are subhyaline to pale olivaceous when young, olive to dark brown at maturity, with 3–5 transverse septa, 1-longitudinal septum in all cells and rarely in end cells. Whereas, *D. aquilaria* has pale grey olivaceous to pale brown conidia with 4–5 transverse septa and 0–2 longitudinal septa (Sun et al. 2017).

Dematipyriforma muriformis shares some similar characteristics with *Neomonodictys* taxa in Pleurotheciaceae, such as monoblastic, integrated, terminal, determinate conidiogenous cells and muriform conidia. *Neomonodictys*, however, lacks sporodochial conidiomata and conidia of *Neomonodictys* are subglobose to globose, while, *Dematipyriforma muriformis* has ellipsoidal to obovoid conidia (Hyde et al. 2020b).

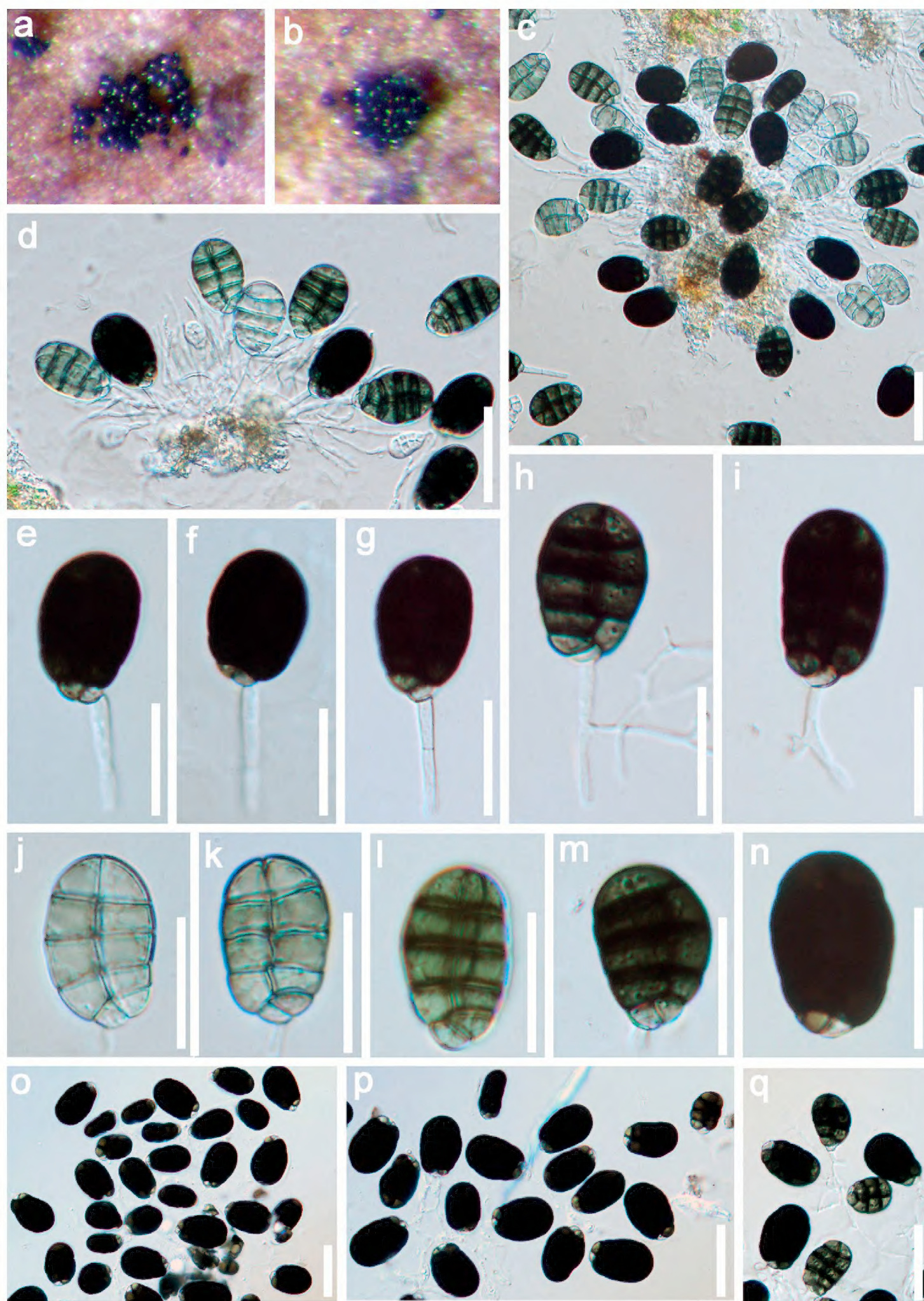


Figure 2. *Dematiopyriforma muriformis* (MFLU 21–0146, holotype) **a, b** colonies on wood **c–d** conidiomata **e–i** conidiophore with conidia **j–q** conidia. Scale bars: 30 μ m (**c–d, o–q**); 20 μ m (**e–n**).

***Dematiapyriforma nigrospora* (Boonmee, D.F. Bao & K.D. Hyde) D.F. Bao, K.D. Hyde & Z.L. Luo, comb. nov.**

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≡ *Rhexoacrodictys nigrospora* Boonmee, D.F. Bao & K.D. Hyde, in Boonmee et al., Fungal Diversity 111: 200 (2021).

Holotype. THAILAND, Phetchabun Province, on decaying bark, 25 July 2019, S. Boonmee, LSP03 (MFLU 21–0073).

Descriptions and illustrations. See Boonmee et al. (2021).

Notes. *Rhexoacrodictys nigrospora* was introduced by Boonmee et al. (2021) based on morphological characters and phylogenetic analysis. In our phylogenetic analysis, *R. nigrospora* clustered with two *Dematiapyriforma* species (*D. aquilaria* and *D. muriformis*) in a distinct clade within Pleurotheciaceae (Fig. 1). Therefore, we transfer *Rhexoacrodictys nigrospora* to *Dematiapyriforma*, as *Dematiapyriforma nigrospora* comb. nov.

Dematiapyriforma nigrospora resembles *D. muriformis* in having micronematous or semi-macronematous, mononematous conidiophores and monoblastic, polyblastic, integrated, terminal conidiogenous cells. However, *D. nigrospora* differs from *D. muriformis* in having brown to dark brown conidiophores and globose to subglobose, dark brown to black conidia (Boonmee et al. 2021). Conidiophores of *D. muriformis* are hyaline and conidia are ellipsoidal to obovoid, muriform, and subhyaline to pale olivaceous when young, olive to dark brown at maturity.

***Rhexoacrodictys fimicola* (M.B. Ellis & Gunnell) W.A. Baker & Morgan-Jones, in Baker, Partridge & Morgan-Jones, Mycotaxon 82: 103 (2002)**

Fig. 3

Holotype. MAYA, Perak, on elephant dung, September 1958, A.H.S, Onions, IMI 76413.

Description. *Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on the substratum superficial, effuse, hairy or velvety, black. *Mycelium* mostly immersed, composed of branched, septate, smooth, pale brown hyphae. *Conidiophores* (17.5–)20–44.5 (–65.5) × 2.5–4.0 µm ($\bar{x}$ = 32.2 × 3.4 µm, n = 20), macronematous, mononematous, erect, straight or slightly flexuous, thick-walled, smooth, orange-brown or brown, 3–7-septate. *Conidiogenous cells* monoblastic, integrated, terminal. *Conidia* 16.5–24 × 11–15 µm ($\bar{x}$ = 20.3 × 13 µm, n = 30), solitary, dry, acrogenous, broadly oval to subglobose, muriform, transversely and longitudinally septate, with transverse septa typically spanning the whole conidial width, with longitudinal septa typically incomplete, short; dark-blackish brown to black, smooth, narrowly truncate at the base.

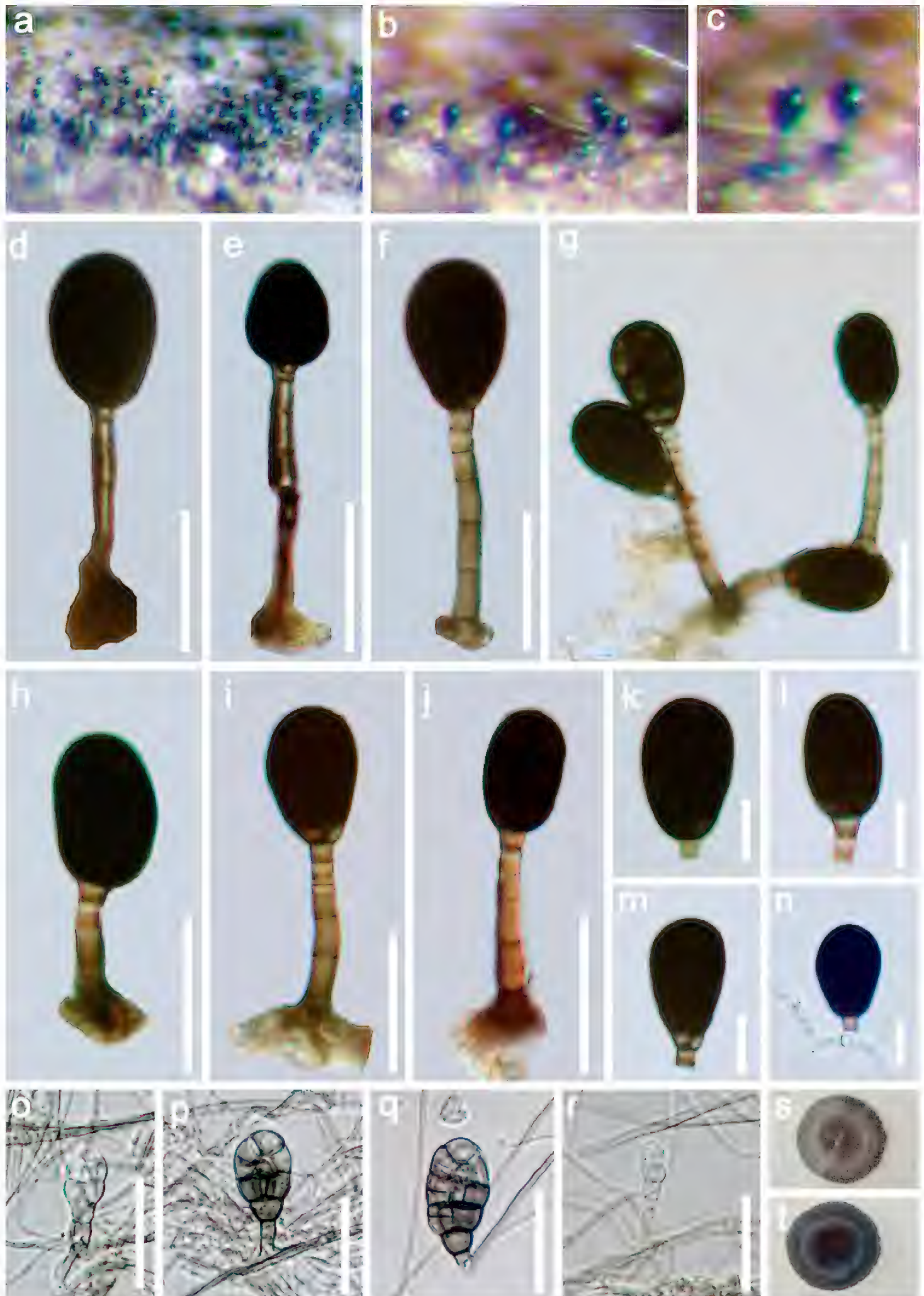


Figure 3. *Rhexoacrodictys fimicola* (MFLU 21-0147, new record) **a–c** colonies on wood **d–j** conidiophores with conidia **k–m** conidi **n** germinating conidium **o–r** re-produced asexual morph of *Rhexoacrodictys fimicola* **s–t** culture on PDA from surface and reverse. Scale bars: 20 µm (**d–j**, **o–r**); 10 µm (**k–n**).

Cultural characteristics. *Conidia* germinating on PDA within 24 h. Germ tubes produced from the basal cell. *Colonies* on PDA reaching 3 cm diameter in 30 days at 20–25 °C, pale brown, with dense, tight mycelia on the surface, sparse at the margin, reverse dark brown, with smooth margin. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, monoblastic, integrated, hyaline to pale brown, smooth. *Conidia* broad oval to subglobose, muriform, strongly constricted at all the septa, hyaline when young, brown to grayish-brown when aged, smooth-walled.

Material examined. THAILAND, Bangkok Province, Bang Kapi District, on decaying wood submerged in a freshwater stream, 3 October 2017, Z.L. Luo, Bsite 4–3–2 (MFLU 21–0147 = KUN-HKAS 122859), living culture, MFLUCC 18–0340.

Notes. In the phylogenetic analysis, our new isolate MFLUCC 18–0340 clustered with three strains of *Rhexoacrodictys fimicola* (HMAS 42882, HMAS 43690 and HMAS 47737) with strong support (100% ML/ 1.00 PP). The nucleotide BLASTn search of ITS showed that our new strain (MFLUCC 18–0340) has 99.7%, 99.3% and 99.1% similarities with strain HMAS 43690, HMAS 47737 and HMAS 42882 of *Rhexoacrodictys fimicola*, respectively. Morphologically, our new collection is similar to *R. fimicola* in having macronematous, mononematous, indeterminate conidiophores, integrated, terminal, monoblastic, pale brown conidiogenous cells and broadly oval to subglobose, transversely and longitudinally septate, smooth, brown to black conidia, with the size of conidia and conidiophores are overlapping (Ellis 1961; Baker et al. 2002). Based on both phylogeny and morphology, we identified our species as *R. fimicola*.

Rhexoacrodictys fimicola was originally introduced by Ellis (1961) as *Acrodictys fimicola*. Baker et al. (2002) transferred *A. fimicola* to *Rhexoacrodictys* based on morphological characteristics. *Rhexoacrodictys fimicola* has been reported on *Bambusa vulgaris* and elephant dung from Africa and Malaysia respectively. Our collection, on the other hand, was collected from freshwater habitats and represents the first time it was reported from Thailand.

***Pleurothecium aquaticum* Z.L. Luo, H.Y. Su & K.D. Hyde, in Luo, Hyde, Bhat, Jeewon, Maharachchikumbura, Bao, Li, Su, Yang & Su, Mycol. Prog. 17(5): 526 (2018)**

Fig. 4

Description. *Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *colonies* on substratum, effuse, shining, dark brown to black. *Mycelium* partly immersed, composed of septate, branched, smooth, dark brown hyphae. *Conidiophores* 84–110 × 3–4 µm ($\bar{x}$ = 97 × 3.4 µm, n = 10), macronematous, mononematous, erect, simple, unbranched, straight or slightly flexuous, 5–8-septate, dark brown, pale towards apex, smooth. *Conidiogenous cells* integrated, polyblastic, terminal, hyaline, denticulate, smooth. *Conidia* 18–22 × 4–5 µm ($\bar{x}$ = 20 × 4.5 µm, SD = 4 n = 30), acrogenous, solitary, clavate, mostly curved, rounded at apex, tapering at base, hyaline, 3-septate, with guttulate cells, smooth.

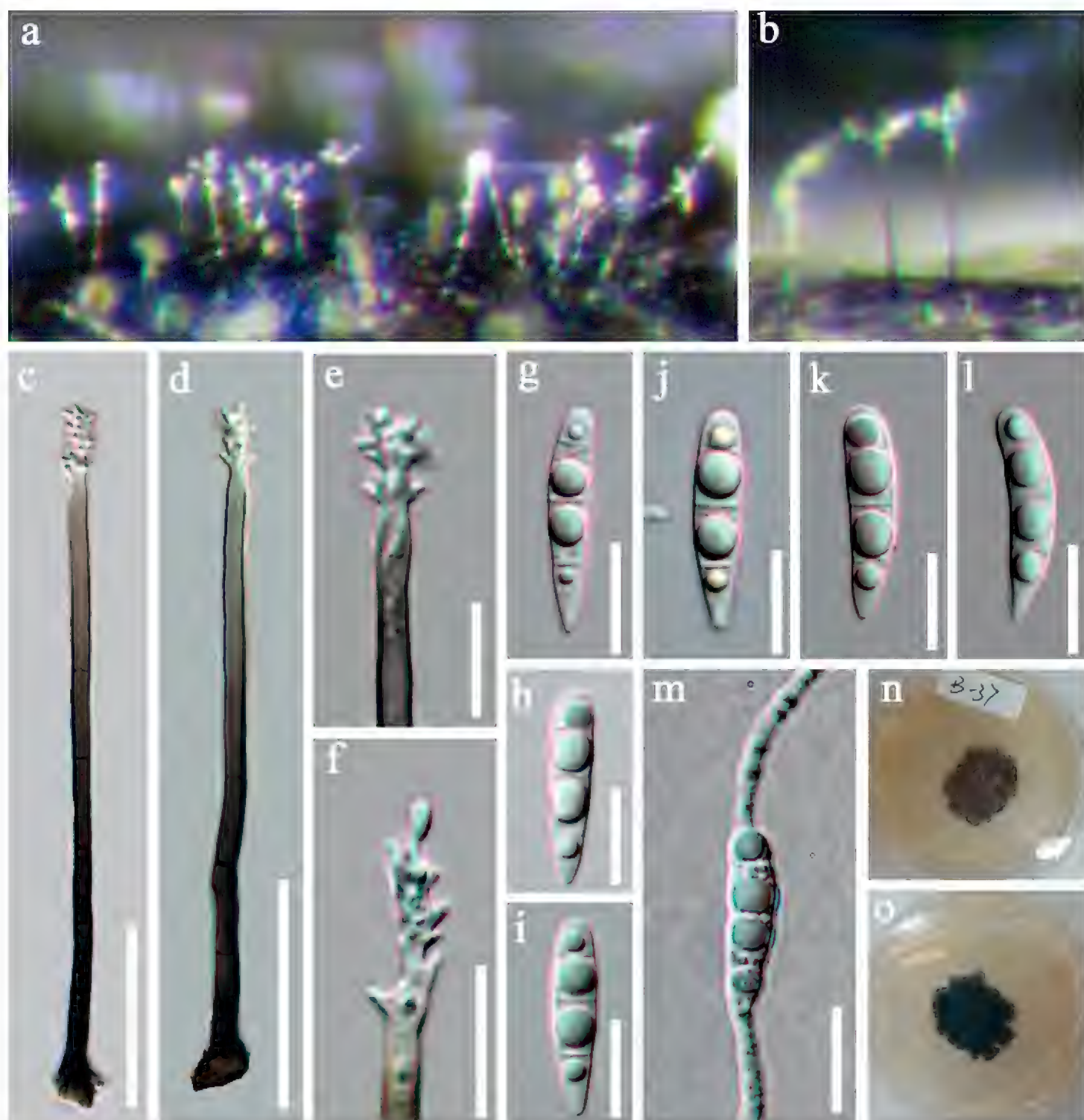


Figure 4. *Pleurothecium aquaticum* (MFLU 21–0148, new record) **a, b** colonies on wood **c, d** conidiophores **e, f** conidiogenous cells **g–i** conidia **m** germinating conidium **n, o** culture on PDA from surface and reverse. Scale bars: 30 μ m (**c, d**); 10 μ m (**e–m**).

Cultural characteristics. *Conidia* germinating on PDA within 24 h. Germ tubes produced from the basal and apical cells. *Colonies* on PDA reaching 2.3 cm diameter in 30 days at 20–25 °C, with dense mycelia, dry, rigid, rugose, dark brown, reverse dark brown.

Material examined. THAILAND, Prachuap Khan, on submerged decaying wood, 15 August 2017, V. Kumar, site1–24–2 (MFLU 21–0148 = KUN-HKAS 122857), living culture, KUNCC 21–0477.

Notes. In the phylogenetic analysis, our new collection KUNCC 21–0477 clustered with the ex-type strain of *Pleurothecium aquaticum* (MFLUCC 17–1331) with high (100% ML/1.00 PP). In addition, the ITS and LSU BLASTn search on NCBI GenBank showed that our new strain is 99.88% and 97.45% similarities to the ex-type

of *P. aquaticum* (MFLUCC 17–1331). The new collection is morphologically similar to *P. aquaticum* in having macronematous, mononematous, septate, brown, pale brown towards the apex conidiophores, integrated, terminal, polyblastic, denticulate conidiogenous cells and hyaline, cylindrical or clavate, rounded at the apex, obtuse and tapering towards base, 3-septate conidia. We therefore identified our new collection as *P. aquaticum*. *Pleurothecium aquaticum* was introduced by Luo et al. (2018a) collected from freshwater habitats in China. Our new collection, on the other hand, was collected from Thailand and is a new record for Thailand.

Discussion

Pleurotheciaceae is a diverse family. The sexual morphs of Pleurotheciaceae are quite similar and difficult to distinguish without molecular data (Réblová et al. 2016; Hyde et al. 2020a). However, the asexual morphs in the family are morphologically diverse. Most genera have mononematous, macronematous conidiophores (*Anapleurothecium*, *Pleurothecium*, *Pleurotheciella* and *Rhexoacrodictys*) (Réblová et al. 2016; Luo et al. 2018a, 2019; Hyde et al. 2020a), whereas some genera have synnematous conidiophores (*Phaeoisaria* and *Phragmocephala*) (Höhnelt 1919; Mason and Hughes 1951; Seifert et al. 2011; Wijayawardene et al. 2012; Su et al. 2015; Réblová et al. 2016; Luo et al. 2018a), and others with micronematous or reduced conidiophores (*Neomonodictys* and *Sterigmatobotrys*). (Hyde et al. 2020b). Conidiogenous cells of *Anapleurothecium*, *Pleurothecium*, *Phaeoisaria* and *Pleurotheciella* are polyblastic and denticulate (Réblová et al. 2012, 2016; Monteiro et al. 2016; Luo et al. 2018a). *Phragmocephala* and *Monotosporella* have monoblastic conidiogenous cells (Mason and Hughes 1951; Hyde and Yanna 2002; Wijayawardene et al. 2012; Su et al. 2015). Conidia of Pleurotheciaceae are diverse in their shape, color and septation. Conidia of *Sterigmatobotrys* are fusiform and in persistent chains (Heuchert et al. 2018); *Helicoascotaiwania* has helicosporous conidia (Dayarathne et al. 2019); *Anapleurothecium*, *Melanotrigonum*, *Pleurothecium*, *Phaeoisaria* and *Pleurotheciella* have clavate, ellipsoidal, obovoidal, fusiform-cylindrical, hyaline or brown, aseptate or transversely septate conidia (Réblová et al. 2012, 2016; Monteiro et al. 2016; Hernandez-Restrepo et al. 2017; Luo et al. 2018a); *Monotosporella*, *Neomonodictys* and *Phragmocephala* have ellipsoidal or subglobose to globose conidia (Mason and Hughes 1951; Hyde and Yanna 2002; Wijayawardene et al. 2012; Su et al. 2015; Hyde et al. 2020b). However, conidia of *Neomonodictys* are muriform (Hyde et al. 2020b), whereas, *Phragmocephala* and *Monotosporella* have transversely septate conidia.

In this study, we introduced a new asexual species, *Dematiapyriforma muriformis* based on both morphology and phylogeny. *Dematiapyriforma* was introduced by Sun et al. (2017) with a single species *D. aquilaria* which was reported as an endophyte from *Aquilaria crassna* in China. While our new species is a saprobe isolated on submerged wood from freshwater habitats in Thailand. In addition, *Rhexoacrodictys nigrospora* is transferred to *Dematiapyriforma* in this study. Currently, three species are accepted in

the genus. Morphologically, the muriform conidia of *Dematipyriforma* are similar to *Neomonodictys*, *Saprodesmium* and *Coleodictyospora*. However, *Dematipyriforma* can be distinguished from *Neomonodictys* by the shape of conidia (ellipsoidal to obovoid vs. subglobose to globose) and conidiophores (semi-micronematous to macronematous vs. micronematous or lacking conidiophores, Hyde et al. 2020b). *Dematipyriforma* differs from *Coleodictyospora* in the conidia lacking a semi-gelatinous sheath (Dong et al. 2021). *Dematipyriforma* is distinct from *Saprodesmium* by the micronematous to semi-macronematous, simple or branched, hyaline, cylindrical, conidiophores, whereas, conidiophores of *Saprodesmium* are micronematous, unbranched, consisted of 1–4 subglobose smooth, hyaline cells (Dong et al. 2021).

Rhexoacrodictys comprises six species of which four species (*R. erecta*, *R. fimicola*, *R. martini* and *R. queenslandica*) have sequence data available in the GenBank. Among them, *R. martini* and *R. queenslandica* were transferred to *Distoseptispora* and *Junewangia* based on phylogenetic analysis (Xia et al. 2017). However, sequence data of *R. martini* are doubted by several studies (Sun et al. 2020; Shen et al. 2021), as its morphology does not fit with the characters of *Distoseptispora*. *Rhexoacrodictys erecta* and *R. fimicola* clustered within Pleurotheciaceae (Luo et al. 2019; Dong et al. 2021). The placement of *Rhexoacrodictys* was questionable since it was established. Baker et al. (2002) established the genus; however, they did not mention the placement of the genus. Xia et al. (2017) firstly provided sequence data for *Rhexoacrodictys erecta* (Type species of *Rhexoacrodictys*) and *R. fimicola* based on their fresh collections, their phylogenetic analysis showed that *R. erecta* and *R. fimicola* clustered within Savoryellaceae. However, they did not include the related orders (Conioscyphales, Fuscosporellales and Pleurotheciales) in Savoryellomycetidae. Luo et al. (2019) found that *R. erecta* and *R. fimicola* grouped in Pleurotheciaceae. Recently, Dong et al. (2021) obtained the same result as Luo et al. (2019). However, Boonmee et al. (2021) and Wijayawardene et al. (2022) placed *Rhexoacrodictys* in Savoryellaceae (Savoryellales). Our result is consistent with Luo et al. (2019) and Dong et al. (2021), the two species clustered within Pleurotheciaceae (Fig. 1). On the other hand, the morphology of *Rhexoacrodictys* is similar to *Dematipyriforma*, *Neomonodictys* and *Saprodesmium*, in having muriform conidia, micronematous conidiophores and holoblastic, monoblastic conidiogenous cells. Therefore, we formally accepted *Rhexoacrodictys* in Pleurotheciaceae (Pleurotheciales) based on morphological characters and phylogenetic analysis.

In our phylogenetic analysis, *Rhexoacrodictys erecta* and *R. fimicola* clustered with *Monotosporella setosa* which is the type species of *Monotosporella*. Morphologically, *R. erecta* and *R. fimicola* fit well within the genus concept of *Monotosporella* in having macronematous, mononematous, brown, septate conidiophores, monoblastic, percurrent conidiogenous cells and acrogenous, brown septate conidia (Hughes 1958; Baker et al. 2002; Hyde and Yanna 2002). However, the strain of *Monotosporella setosa* (HKUCC 3713) lacks a morphological description. Therefore, further study is necessary to clarify the relationship between *Rhexoacrodictys* and *Monotosporella*, whether they should be combined into one genus or not. In addition, our phylogenetic analysis showed that three strains of *R. erecta* clustered with *Monotosporella setosa*. However,

M. erecta differs from *M. setosa* in having transverse and longitudinal septation, while, conidia of *M. setosa* only have transverse septa. Only LSU sequence data is available for *M. setosa*, which is not significant to distinguish in the phylogenetic tree, but morphologically they are quite distinct. Hence, we maintain them as two distinct species, however, further morphological and phylogenetic analysis is required to clarify the relationship between these two species.

In our phylogenetic analysis, *Pleurothecium obovoideum* was placed distant from *Pleurothecium* and close to *Neomonodictys muriformis* and *Coleodictyospora muriformis* which is consistent with recent studies (Luo et al. 2018a, 2019; Hyde et al. 2020b). *Pleurothecium obovoideum* was introduced by Arzanlou et al. (2007) based on morphological characters. However, their analysis showed that *P. obovoideum* clustered with *Ascotaiwania hughesii* and with more sequence data now available for *Pleurothecium* species, *P. obovoideum* is shown phylogenetically distinct from *Pleurothecium*. Morphologically, *P. obovoideum* is similar to *Pleurothecium* in having distinct brown conidiophores, polyblastic, denticulate conidiogenous cells and pale brown, ellipsoidal to obovate conidia. However, conidia of *P. obovoideum* are aseptate and solitary or in short chains whereas the conidia of *Pleurothecium* are solitary and unicellular or septate. Thus, the placement of *P. obovoideum* needs revision in the future with more evidence.

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